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DOES THE DIAMETER OF THE TITANIUM ORTHOPAEDIC SCREW INSERTION HOLLOW INFLUENCE THE MECHANISM OF CONTACT OSTEOGENESIS ? A COMPARATIVE ASSESSMENT IN THE FEMALE RABBITS' FEMORAL BONE

DIAMETRUL ORIFICIULUI DE INSERȚIE A ȘURUBULUI ORTOPEDIC DIN TITAN INFLUENȚEAZĂ MECANISMUL OSTEOGENEZEI DE CONTACT ? O EVALUARE COMPARATIVĂ LA NIVELUL OSULUI FEMURAL AL FEMELELOR DE IEPURE

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ABSTRACT

The surgical insertion of an orthopaedic screw leads to bone trauma that will force the osseous tissue to regenerate. The cascade of events that follow is directly influenced if the insertion is done under conditions where excessive pressure is exerted on the implantation area. The aim of the present study was to determine if the diameter of the insertion hollow significantly influence the mechanism of contact osteogenesis after the insertion of the titanium orthopaedic screw. The implants were inserted in the femoral bone of female rabbits in 1.8 mm hollow (group 1) or 1 mm hollow (group 2). After 6 weeks from the intervention, histological and morphometric assessments were done at the implantation site. The results suggested significant differences both in terms of the amount of bone proliferated on the interface and in terms of the maturation stage it reached. In the first group, the layer of bone deposited on the interface is relatively thick (291389.84 μm^2), but the thickness is not uniform throughout the interface, while in the second group, the bone proliferated extends over the entire interface but is significantly thinner (47613.62 μm^2). Therefore, the space that remains between the surface of the screw and the wall of the cortical bone hollow must be of a certain size to ensure the best conditions for the rapid and proper development of repair processes through contact osteogenesis.

Keywords: contact osteogenesis, orthopaedic implant, morphometry

REZUMAT

Inserarea chirurgicală a unui șurub ortopedic duce la traumatisme osoase care vor forța țesutul osos să se regenereze. Cascada evenimentelor care urmează este direct influențată dacă inserția se face în condiții în care se exercită o presiune excesivă asupra zonei de implantare. Scopul prezentului studiu a fost de a determina dacă diametrul orificiului de inserție influențează semnificativ mecanismul osteogenezei de contact după introducerea șurubului ortopedic de titan. Implanturile au fost introduse în osul femural al femelelor de iepure în orificii cu diametrul de 1,8 mm (lotul 1) sau diametrul de 1 mm (lotul 2). După 6 săptămâni de la intervenție s-au făcut evaluări histologice și morfometrice la locul de implantare. Rezultatele au sugerat diferențe semnificative atât în ceea ce privește cantitatea de os proliferată pe interfață, cât și în ceea ce privește stadiul de regenerare la care a ajuns. În primul grup, stratul de țesut osos depus pe interfață este relativ gros (291389,84 μm^2), dar grosimea nu este uniformă pe toată interfața, în timp ce la al doilea lot, osul proliferat se extinde pe întreaga interfață, dar este semnificativ mai subțire (47613,62 μm^2). Așadar, spațiul care rămâne între suprafața șurubului și peretele orificiului de inserție trebuie să fie de o anumită dimensiune adaptată la diametrul șurubului pentru a asigura cele mai bune condiții pentru desfășurarea rapidă și corectă a proceselor de reconstrucție prin osteogeneză de contact.

Cuvinte cheie: osteogeneză de contact, implant ortopedic, morfometrie

As a result of the trauma that accompanies the surgical act necessary to insert an orthopaedic screw, a discontinuity is created in the bone, and bone necrosis will occur about 1 mm from the surface of the screw (2, 18). Necrosis is a consequence of rupture of blood vessels in the area, followed by ischemia and a lack of oxygen supply to osteocytes that, in living bone, are no more than 0.1 mm away from an intact capillary (5). In the affected area, osteocytes die, leaving the lacunae empty. These lesions normally accompany the insertion process of orthopaedic screws, but they can be amplified if the insertion is done under conditions where excessive pressure is exerted on the implanta-

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tion area. Moreover, some authors argue that compressive forces are beneficial up to a certain level, but excessive ones can cause significant damage to the bone through the appearance of extensive bone resorption and even loss of implant anchoring (1).

The healing of peri-implant sites is a complex process that sums up a cascade of cellular and extracellular events. Healing is influenced by many factors, such as bone type (cortical or trabecular), implant location, severity of trauma at the implantation site, mechanical stability, species, and age. In all cases, the result of healing aims to restore tissue continuity. This complete reconstitution is a unique feature of bone in the adult animal, while all the other tissues are healing with the participation of connective tissue (5). Peri-implant bone healing includes two processes represented by contact osteogenesis and remote osteogenesis. Contact osteogenesis is the preferred form of bone proliferation and is characterised by bone proliferation directly on the implant surface. In this form of osteogenesis, the potentially osteogenic cell population migrates through the fibrin network of the clot formed around the implant and reaches its surface. Here they begin the synthesis of a non-collagenous material that is deposited on the surface of the implant, forming the so-called cement line. This cement line mineralizes, and collagen bone matter is deposited over it, layer by layer, giving rise to tissue bone (with a plexiform disposition) (5). Remote osteogenesis is characterized by the fact that cells with osteogenic potential do not reach the implant surface, and the first bone material is deposited on the periphery of the bone defect. Through further proliferation, it advances towards the implant surface (4, 11).

MATERIAL AND METHODS

Biological material

Ten domestic rabbits (*Oryctolagus cuniculus*), females, 12 months old, with an average weight of 4 kg, were used for the study. The animals were divided into two groups [n = 5/Group 1 (G1); n = 5/Group 2 (G2)].

Non-biological materials

The design of the experiment implied the use of titanium screws with a diameter of 2 mm, drills with a diameter of 1.8 mm, and drills with a diameter of 1 mm. The substances used for the surgical intervention were represented by xylazine, ketamine, enrofloxacin, and meloxicam.

Ethics

The experimental design and interventions on the animals were approved by the Institutional Bioethics Committee with no. 289/03.06.2023, by the National Veterinary Sanitary and Food Safety Authority (no. 384/20.08.2023), and are in accordance with national legislation (Law 43 of 2014) and European legislation (EU Directive 63 of 2010).

Experimental intervention

Animals in both groups (G1, G2) were anaesthetized by intramuscular administration of xylazine (5 mg/kg) and ketamine (40 mg/kg). In order to reach the femoral bone, the intervention area was mechanically and chemically prepared, followed by the incision of the skin and muscles. In G1, a hole was performed with a drill with a diameter of 1.8 mm, while in G2, the drill's diameter was 1 mm. Because the screws were designed to penetrate by self-tapping, they were inserted by manual screwing. After the insertion, the suture of the tissues in the intervention area was performed. Post-operative treatment was represented by the administration of enrofloxacin SC (20 mg/kg) for 5 days and meloxicam SC (1 mg/kg) for 3 days. The animals were euthanized after 6 weeks, and the portion of the femur containing the implant was harvested.

Histological assessment

The harvested pieces were fixed in 10% buffered formalin for 7 days, then decalcified with trichloroacetic acid and included in paraffin. The next step was represented by the sectioning of the tissues (5 micrometres in thickness) and staining the sections with Goldner's trichrome method. The histological slides were examined under an Olympus BX41 microscope, and an Olympus E-330 digital camera was used to capture microscopic images.

Morphometric evaluation

The morphometric assessment of the bones was performed using ToupView software. According to the diameter of the hole created in the bone, the animals were divided into two groups: G1 (1.8 mm hole) and G2 (1 mm hole). The bone surface (μm^2; n=7/group) was measured from the best fit intercepted by the histological sections.

Statistical analysis

The data obtained after the morphometric assessment were statistically analysed using GraphPad PRISM 8.0.1 software and Microsoft Excel 2016. To compare and interpret the mean surface of the proliferated bone for the two groups, descriptive statistics were calculated, followed by a normal distribution and a t-test (unpaired, two-tailed).

RESULTS AND DISCUSSION

On the portion of the interface next to the wall of the cortical bone, respectively, the one between the periosteum and the endosteum, the difference is great

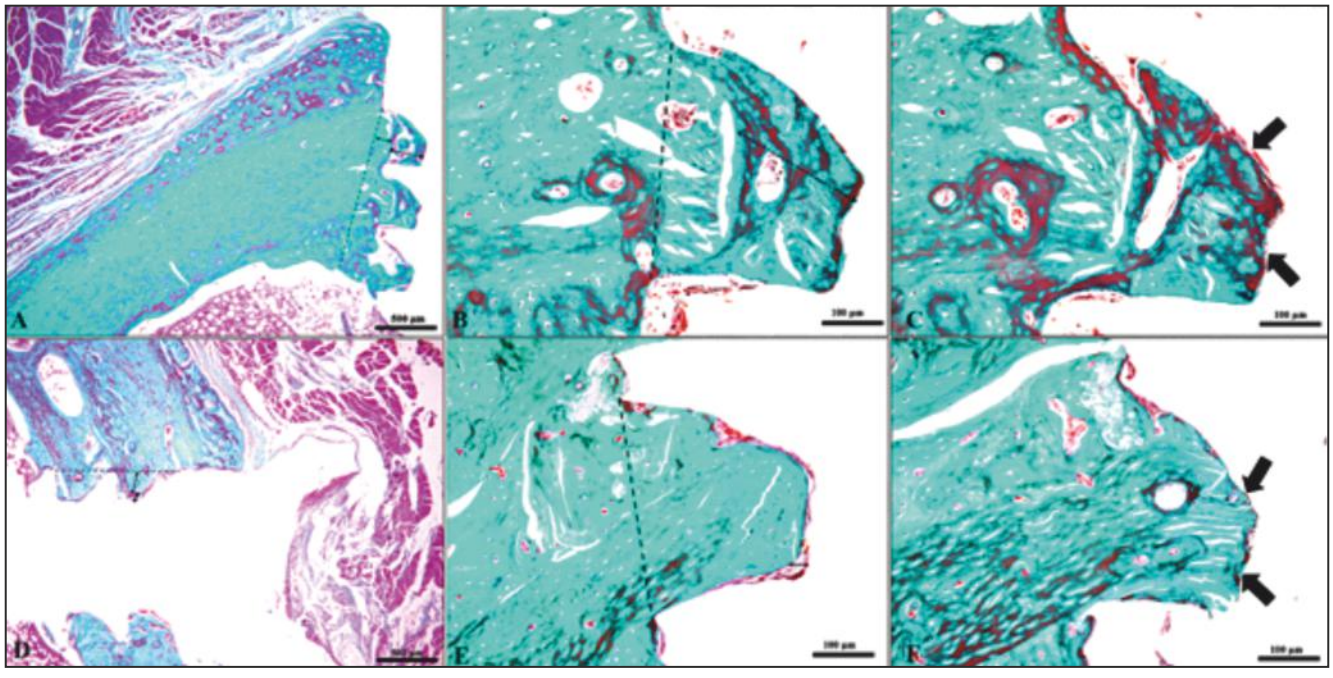

Fig. 1. Bone proliferation at the level of the screw insertion area; **A**- overview of the proliferation zones, with the appearance of saw teeth (G1); **B**- the newly proliferated bone, thicker at the level of the groove between the implant turns (double arrow) (G1); **C**- the plexiform organisation of the newly proliferated bone (arrows) (G1); **D**- overview of the proliferation zones, with the appearance of saw teeth (G2); **E**- a thin and relatively uniform layer in thickness throughout the interface, without significant differences between that proliferated in the grooves between the turns and that covering the turns (G2); **F**- the thin layer of newly proliferated bone, mostly in the cement line stage, which, only in places, appears to be covered by a small amount of osteoid (arrows) (G2)

between the two experimental groups, both in terms of the amount of bone proliferated on the interface (Figures 1B and 1E) and in terms of the stage it reached in the 6 weeks elapsed since the insertion of implants (Figures 1C and 1F).

In the first group, where the insertion of the implant was realised into the hole of 1.8 mm (G1), the amount of newly proliferated bone is clearly higher and its arrangement somewhat particular (Figures 1A, 1B, and 1C). The layer of bone deposited on the interface is relatively thick, but the thickness is not uniform throughout the interface next to the wall of the cortical bone. More specifically, the newly proliferated bone is significantly thicker at the level of the groove between the implant turns compared to the one next to the spires, so that overall, the bone proliferated here has the appearance of saw teeth (Fig. 1A). As a stage of organisation, the bone proliferated here is for now in the stage of bone tissue (plexiform) for the most part, but also in areas limited by osteoid (Fig. 1 C). In the second group, where the insertion of the implant was realised into the 1 mm hole, the situation is very different in the sense that the bone proliferated here, even if it extends over the entire interface, is significantly thinner compared to the other experimental group (Figures 1D, 1E, and 1F). It is represented by a thin and relatively uniform layer in thickness throughout the interface, without significant differences between that proliferated in the grooves between the turns and that covering the turns (Fig. 1 E). In terms of organisation, this thin layer of newly proliferated bone is mostly in the cement line stage, which, only in some places, appears to be covered by a small amount of osteoid (Fig. 1F).

Table 1

Comparative morphometric features regarding the newly proliferated bones in the screw turn

	Mean surface of the total bone from the groove (μm^2)	Mean surface of proliferated bone (μm^2)	Mean surface of old bone in the groove (μm^2)	% of newly proliferated bone (average)	% of old bone in the groove (average)
Group 1	494553.796	291389.842	203163.954	58.685	41.314
Group 2	406662.742	47613.628	359049.113	12.740	87.259

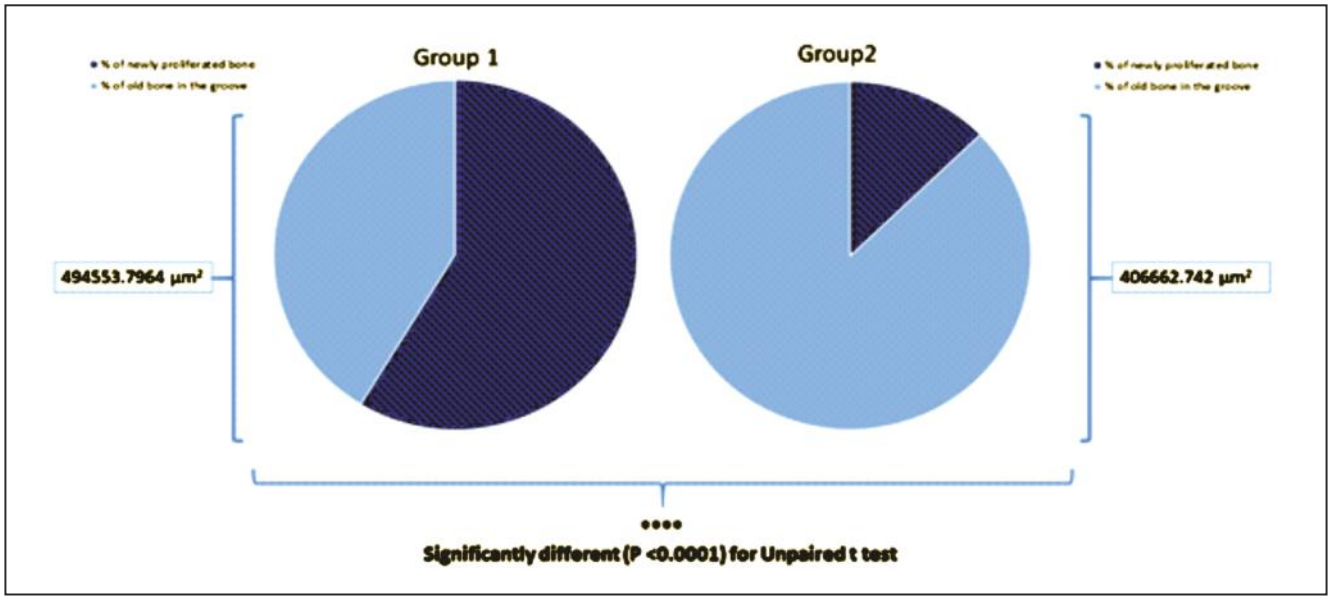

Fig. 2. Graphical representation of the differences in proliferation between the two experimental groups

Morphometric evaluation suggested that the mean surface of the bone from the groove had similar values (494553.796 μm^2 for the G1 – 1.8 mm drill, 406662.742 μm^2 for the G2 – 1 mm drill) (Table 1). However, the mean surface of the proliferated bone registered significant differences between the two groups (291389.842 μm^2 for the G1 (n = 7) – 1.8 mm drill vs. 47613.628 μm^2 for the G2 (n = 7) – 1 mm drill), as demonstrated by the t-test ($p<0.0001$). In other words, the % of old bone in the groove is 41.314% for G1, while for the G2, it represents a higher surface - 87.259 % (Fig. 2).

The bone repair process is complex and involves a cascade of cellular and molecular interactions leading to stem cell differentiation, osteoblast recruitment, and mineralized matrix production (9). The regeneration of the bone around the implant is a process similar to, but not identical to, bone regeneration after fractures. Through a complex process, a mechanically and chemically stable implant is gradually fully incorporated into the bone (17). In the first phase, the implant surface comes into contact with the blood, at which point ion exchange and protein absorption begin. Blood platelets release cytokines and growth factors involved in osteogenesis to the implant surface, such as platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β) (16). They act as chemotactic factors, with the role of provoking the migration of osteoprogenitor cells from the marrow and blood supply to the implant surface (7). In addition, platelets initiate the cleavage of fibrinogen into fibrin, forming a fibrin-platelet aggregate that will be absorbed on the implant surface (8). Guided by chemotactic signals, osteoprogenitor cells migrate to the implant surface using the fibrin network of the blood clot. Normally, osteoprogenitor cells reach the implant surface, where they differentiate into osteoblasts and initiate bone proliferation directly on the implant surface, known as contact osteogenesis. The first tissue that occurs during contact osteogenesis is the cement line, that is deposited as a non-collagenic layer interposed between the implant surface and the collagen component of the new bone. Contact osteogenesis is the preferred form of bone proliferation in the case of the osseointegration of implants. Next comes the mineralization of the cement line with the help of proteins that are responsible for organising hydroxyapatite crystals. In this context, the mineralized cement line intimately follows the surface contour of the implant, so that the bone attachment mechanism is one of micro-mechanical interdigitation of bone tissue with the implant surface (6, 10, 20). Other authors state that micromechanical interdigitation of the cement line with the substrate is found both at natural remodelling sites and on biomaterial surfaces (4). Between the collagen compartment and the implant surface, the calcified cement line is interposed so that the implant surface does not come into direct contact with collagen (3). Other authors state that there is little evidence to support the claim that between the first identifiable collagen fibre and the implant surface, a collagen-free area is present. They consider it likely that the lack of collagen fibrils in the immediate vicinity of the implant surface is only a consequence of spatial constraints (22).

The next stage is the formation and deposition of the osteoid, a stage in which osteoblasts synthesise collagen precursors that are assembled extracellularly. In this way, the new bone matrix appears, which is deposited rapidly and has a less ordered structural organisation (plexiform aspect). By mineralizing collagen, the primary bone is formed, whose strength is obviously higher, so it provides support for both compression and shear loads. The next step is to reshape the primary bone to replace

this originally deposited bone with a stronger one. When remodelling is completed, and in the case of bone proliferating on the interface and in the depth of the interface, the boundary between them disappears, the bone reaches homeostasis, and the strength of the bone-implant interface reaches equilibrium (11). Remodelling processes are constantly taking place in the body, on the one hand, to replace worn bone components and, on the other hand to adapt the architecture of the bone in relation to the stresses to which it is exposed (12, 13, 26). Following the development of bone proliferation processes on the bone-implant interface of the femoral bone of rabbit females 6 weeks after the insertion of titanium screws, we found the presence of newly proliferated bone on the entire surface of the interface between the periosteum and endosteum, but with large differences between the two experimental groups.

In the case of the version with a 1.8 mm hole, the newly formed bone is arranged in the form of a continuous layer, but with large differences in thickness from one area to another. The greatest thickness of newly proliferated bone is present in the grooves between the implant turns, while over the top of the turns, the layer of newly proliferated bone tissue is much thinner. This significant difference in thickness has only one explanation, namely the fact that at the time of screw insertion, the tip of the coils comes into intimate contact with the bone while the deep half of the grooves does not. There remains a space between the surface of the implant and the bone wall, a space in which a well-represented blood clot form. The situation near the coils is completely different, where the space between the surface of the implant and the bone wall is very small, so that the newly proliferated tissues occupy only this space and will only be able to expand when the remaining bone in the vicinity is gradually lysed to make room for the newly proliferated ones. From a structural point of view, newly proliferated bone is represented by primary bone that contains areas of osteoid in places. It should be noted that the spaces of this bone contain small-calibre blood vessels, which suggests that it is very well vascularized and consequently has ideal conditions for further proliferation. These aspects were also pointed out by other authors, who found that the proliferated bone on the interface does not have the same thickness, being preferentially deposited at the level of the grooves between the turns, where it forms an obviously thicker layer than on the surface of the turns (19). Other researchers reached the same conclusion and gave an explanation for this phenomenon, which according to them would be due to the increase in the concentration of platelets in the fibrin network, better represented at the level of the grooves (14, 15). In the case of the 1 mm hole version, during the self-drilling process, the screw wire dug into the bone in such a way that there was practically a very narrow gap between the surface of the screw and the bone. This very small space was then flooded with blood, which organised itself into a clot with the help of the fibrin network. In this narrow space, the thickness of the fibrin lattice was very small, and the number of platelets arriving here was relatively small. Moreover, considering the way the screws were inserted, there was practically no difference in the thickness of this limited space near the grooves compared to the one next to the turns, there being practically a narrow and comparable thickness space throughout the bone-implant interface next to the cortical bone wall. In this limited space, conditions for the proliferation of new bone were very particular. For the proper conduct of contact osteogenesis, certain conditions are necessary that have largely not been met here. Space being limited, the clot was also limited in quantity, and consequently, the fibrin network was also slightly expanded. Under these conditions, the number of platelets was limited, and consequently, the number of factors released from them was small. Added to this was the fact that to reach the surface of the screw, osteogenic cells must be able to migrate easily through the fibrin network of the clot, but here the migration conditions were modest. Under these particular conditions, the proliferation of new bone on the central area of the interface proceeded with some delay and difficulty.

If we compare the newly proliferated bone on the interface in the two experimental groups, we find that in the 1.8 mm hole version, the amount of new bone proliferated by contact osteogenesis is significantly higher than in the 1 mm hole version. Since in the experimental protocol, the only difference was the different diameter of the insertion hole, we can easily conclude that the space that remained between the surface of the screw and the drilled bone was the decisive factor that determined the appearance of such large differences.

This space is not indicated to be larger than 150 µm because there is a risk of scar connective tissue forming instead of bone (25). Other authors state that even full bone coating (a bone-implant contact ratio of 1) is not the ideal situation because there is a risk of depositing a small amount of bone on the interface (11).

The ratio between the diameter of the insertion hole and the total diameter of the screw must be synchronised so that the anchorage is effective but not forced. In order for stress on the bone not to exceed its strength, forces must be applied and evenly distributed along the entire length of the screw (11). The spaces necessary for the proliferation of bone structures must be at least a few micrometres to accommodate small capillaries, tens of micrometres to accommodate entire cells (21, 24), considerably larger to allow lamellar bone formation, and larger to allow osteon development. Thus, pores with dimensions below 50 µm allow the formation of tissue bone (plexiform) (23), those over 100 µm allow the formation of lamellar bones, and those over 140 µm allow the formation of osteons (22).

CONCLUSION

The space that remains between the surface of the screw and the wall of the cortical bone hole must be of a certain size to ensure the best conditions for the rapid and proper development of repair processes through contact osteogenesis. It should not be too large because there is a risk of connective tissue proliferation, but not too small because it does not allow the organisation of a blood clot large enough to ensure easy migration of cells with osteogenic potential to the surface of the screw or a sufficient number of platelets to release growth factors. In addition, this small space does not allow easy expansion of the newly formed bone, so it is deposited in a thin layer as a cement line and has practically no space to deposit new layers. Under these disadvantageous conditions, contact osteogenesis is significantly more modest when space is limited than when adequate space remains between the surface of the screw and that of the cortical bone for organising the clot, migrating cells through it, and depositing newly proliferated bone. The forced insertion of titanium screws is disadvantageous not only because of the pressure they exert on the bone wall but also because they do not provide the necessary space for the proper unfolding of contact osteogenesis.

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